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ONE LINE (ONE DIMENSION) IMAGE ANALYSIS

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ABSTRACT

Traditional pointcounting and linear intercept technics on one side and automatic image analysis on the other reflect the wide range of alternatives in morphometry. At the moment the memory and the speed of microcomputers are limiting factors in practical applications. This applies to two-dimensional analysis of tv-images. There is an area between pointcounting and image analysis applicable to microcomputer: analysis of one dimension. This method is theoretically equivalent with the others but has been too tedious for manual morphometry and is almost neglected in automatic image analysis. Increased memory and the use of 16-bit microcomputers offer further possibilities for modular programming in which test modules and statistical modules can instantly be reprogrammed to modify the type of analysis.

Microcomputing applied to morphometry offers a variety of possibilities limited by human resources and the financial situation. The clinical use of any system today is mainly dependent on software and price factors.

Applied morphometry can be described in two general categories and three different levels of automation (Table 1.)

The two general categories are:

A. Morphometry yielding intrinsic parameters unique to the test situation.

B. Statistical approach evaluating these parameters with each other and with the provided extrinsic parameters (normal values etc.).

The levels of automation in both these categories are:

1.A. Manual methods which could be illustrated by manual feeding of formulas and tables into the computer. As few manually entered paramaters as possible must be selected or

TABLE 1. GENERAL CATEGORIES AND LEVELS OF MORPHOMETRY AND APPLIED STATISITICS.

	Categories	
	A. Morphometry	B. Applied statistics
Levels	:	:
1.Manual control -use of data base management and word processing	: Input of morphometric data	: Input of statistical methods and retyping the data for statictics
2.Advanced desktop aids for data manipulation	: Visual control of input data	: Programmed statistical packages
3.Adjustment of test situation for automation	: Electronic input of screen data with instant links to statistics	: Programmed statistical macros and library programmes

the manual entry should be combined with the normal office routines, which are done anyway. There are many program products available for substituting formular filling and gaining access to data manipulation and statistical analysis. These are available in data base management systems (DBMS) which combined with some office typewriting programs, as word processing systems (WP) offer valuable aids to morphometry.

1.B. To my knowlegde no statistical packages for 1.A.-category approach are available, which means that these must be produced the "hard way". This implies learning of some high level programming languages and advanced understanding how computer works. Certain level of standardization has been reached by applying computer operating systems (CP/M, MS-DOS) and high level languages (Basic etc) to develop and adapt available resources.

2.A. Visual and manual processing of images ("semiautomatic", whatever it means) is generally understood as an interim on a way to fully automatic analysis of morphometric

data. New vistas are possible by a translation of the planar (two dimensional) measurement into a linear (one dimensional) measurement. In the old days the linear measurements were considered time consuming. To the human eye the counting of points (dimensionless morphometry) is as easy as the pointing of structure borders intersecting a line (one dimensional morphometry). Thus in contrast of drawing the outlines of the object (two dimensional morphometry), pointing of objects is fast and accurate and more fields can be analysed by line pointing than drawing the contour within certain period of time.

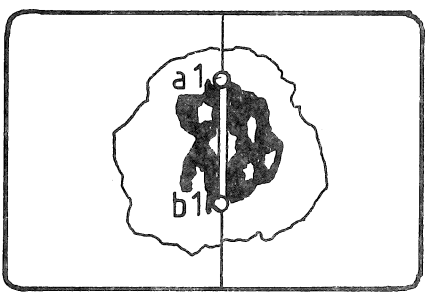
A simple test system of this kind would consist of a microscope, TV-camera, a digitizing microcomputer system and a lightpen. The morphometric probe consists of a test line on the screen (L_T), x,y-coordinates of pairs of points on the test line (the beginning and endpoints of a structure on test line) and number of individual structures per test line. A program can simply produce linear intercepts (L_L), and test-line lengths (L) and number of structures per test line (N_L). Statistical analysis can be added to this.

Example. Simple test situation.

HE stained section for nuclear chromatin estimation based on linear analysis.

The microscopic image is displayed on a tv-screen with a test line. A lightpen for pointing x,y-coordinates is available. Test line length is estimated by micrometry. The testline intersects nuclear borders at points ai and bi.

V Test line



The observer points these with a lightpen and the program calculates intersection points and the length of (ab)i which is linear intercept. Under visual control it is further possible to count structures per test line.

The microcomputer processes the parameters during microscopy and evaluates these sequentially to the given significanse level.

2.B. Statistical program systems have been developed for mathematical and socio-economical purposes. Statistical methods are available for microcomputers, but parameter feeding from the test situation into the statistical program is difficult. Thus programming is required.

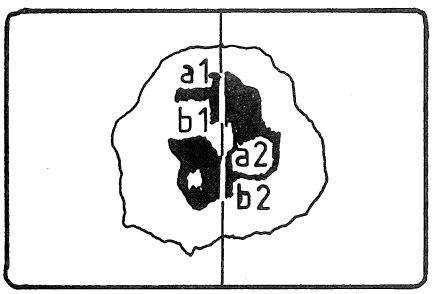
3.A. Full two (or three) dimensional image analysis can be performed by a microscope, a TV-camera and a computer.This is available for basic research and requires skilled technical support, which is hardly available in the clinical situation today. This suggests that one- dimensional approach is the method of choice for the clinical situation.

A test system of this kind would consist of scanning of a test line on the screen. A binary image is prooduced and linear erosions and dilatations performed. The intercepts and line lengths are produced. Numerical analysis would be limited to convex objects only. Combination of visual and automatic analysis could offer a fast multidimensional image analysis for clinical test situations.

Example. Simple test situation.

HE stained section for nuclear chromatin estimation based on linear binary image analysis.

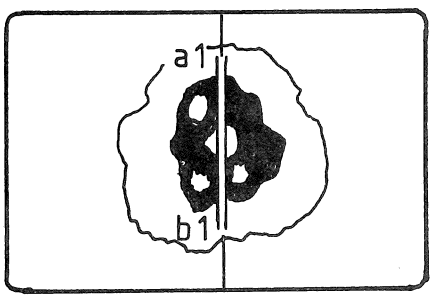
V test line



One line (test line) from the microscopic tv screen can be adjusted to measure binary image. The test line length is measured by micrometry.

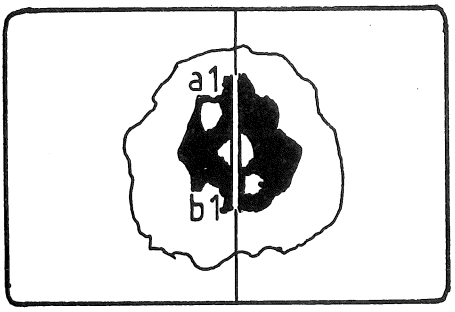
A raw picture captures all different chromatin islands on the test line (a..b)1, (a..b)2, etc. Analogically to previous example volumetric and surface profiles for chromatin can be estimated instantly.

after dilatation:



In dilatation a constant number of bits are added to every endpoint of objects on the test line. Chromatin islands close to each other will coalesce. An enlarged continuous line will emerge.

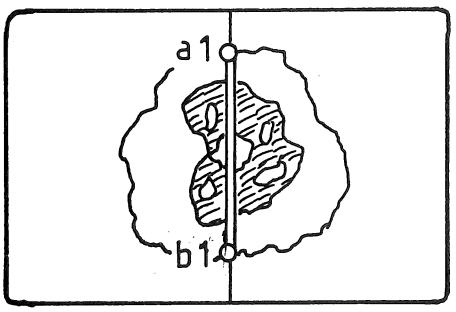
after erosion:



Erosion is reverse to dilatation. From the endpoints of the line image the same amount of bits is substracted. There will be no endpoints within the coalescend lines. Thus the new line can be used for estimation nuclear volume and surface area densities.

Estimates for chromatine coarsity can be produced when data from chromatin aggregates and whole nuclei are combine.

point pointing of cell borders:



The test facilities can further be extended with a lightpen. Low contrast images can be pointed with lightpen to distinguish cell borders from the test field in which automatic nuclear analysis was carried out.

So cell volume and surface densities can be estimated from the same test fields.

Further numerical parameters of nuclear or cellular structures can be entered manually. This test situation will allow fast multidimensional input which combined with a sequential type of statistical analysis will optimize number of necessary manipulations on the screen to the predefined precision.

3.B. Modern operating systems (Unix) and high level languages (C- language) allow building of modules. Morphometric and statistical modules can be linked with the requirements of the actual test situation with the minimum of programming effort.